

## WE CLAIM:

1. A modified L49 sFv, wherein the modification is an amino acid substitution at position 85 (Kabat position H82B) of phenylalanine for serine, threonine or 5 alanine of SEQ ID NO:2.
2. A modified L49 sFv, wherein the modification is an amino acid substitution at position 95 (Kabat position H91) of asparagine for tyrosine or phenylalanine of SEQ ID NO:2.
- 10 3. A modified L49 sFv, wherein the modification is an amino acid substitution at position 85 (Kabat position H82B) of phenylalanine for serine, threonine or alanine, and at position 95 (Kabat position H91) of asparagine for tyrosine or phenylalanine of SEQ ID NO:2.
- 15 4. A modified L49 sFv, wherein the modification is an amino acid substitution at position 40 (Kabat position H39) of lysine for glutamine, and at position 95 (Kabat position H91) of asparagine for tyrosine or phenylalanine of SEQ ID NO:2.
- 20 5. A modified L49 sFv, wherein the modification is an amino acid substitution at position 40 (Kabat position H39) of lysine for glutamine, and at position 85 (Kabat position H82B) of phenylalanine for serine, threonine or alanine of SEQ ID NO:2.
- 25 6. A modified L49 sFv, wherein the modification is an amino acid substitution at position 40 (Kabat position H39) of lysine for glutamine, at position 85 (Kabat position H82B) of phenylalanine for serine, threonine or alanine, and at position 95 (Kabat position H91) of asparagine for tyrosine or phenylalanine of SEQ ID NO:2.
- 30 7. The modified L49 sFv according to claim 1, 2, 3, 4, 5 or 6, which is fused via a peptide bond to a therapeutic agent.
8. The modified L49 sFv according to claim 7, in which the therapeutic agent is a cytotoxic molecule or a pro-drug converting enzyme.

9. The modified L49 sFv according to claim 8, in which the cytotoxic molecule or pro-drug converting enzyme is fused via a peptide bond to the N-terminus of said modified L49 sFv.

5 10. The modified L49 sFv according to claim 8, in which the cytotoxic molecule or pro-drug converting enzyme is fused via a peptide bond to the C-terminus of said modified L49 sFv.

10 11. The modified L49 sFv according to claim 8, in which the cytotoxic molecule is selected from the group consisting of abrin, ricin A, bryodin, pseudomonas exotoxin, diphtheria toxin, saporin, and a porin protein.

12. The modified L49 sFv according to claim 8, in which the pro-drug converting enzyme is selected from the group consisting of *Herpes simplex* thymidine kinase, bacterial cytosine deaminase, cytochrome p450 NADPH oxidoreductase, alkaline phosphatase,  $\alpha$ -galactosidase,  $\beta$ -galactosidase, aminopeptidase, aryl sulfatase, glucose oxidase, caspase, carboxylesterase, xanthine oxidase, elastase, nitroreductase, carboxypeptidase G2, beta-glucuronidase, penicillin-V-amidase, penicillin-G-amidase,  $\beta$ -lactamase,  $\beta$ -glucosidase, and carboxypeptidase A.

20

13. The modified L49-sFv according to claim 8, in which the pro-drug converting enzyme is beta-lactamase.

14. The modified L49 sFv according to any one of claims 1-13, in which the modified L49-sFv is purified.

15. The modified L49 sFv according to claim 1, 2, 3, 4, 5 or 6, which is conjugated to a therapeutic agent.

30 16. The modified L49 sFv according to claim 15, in which the therapeutic agent is a cytotoxic molecule or a pro-drug converting enzyme.

17. A pharmaceutical composition comprising (a) a purified, modified L49 according to any of claims 7-13; and (b) a pharmaceutically acceptable carrier.

35

18. A nucleic acid comprising a nucleotide sequence encoding the modified L49 sFv according to any of claims 1-6.

19. A nucleic acid comprising a nucleotide sequence encoding the modified 5 L49 sFv according to any of claims 7-13.

20. A nucleic acid comprising:

- (a) the nucleotide sequence of SEQ ID NO:1 in which nucleotide residues 253-255 are changed to AGT, AGC, TCT, TCC, TCA, TCG, ACC, ACA, ACG, ACT, 10 GCC, GCA, GCG or GCT;
- (b) the nucleotide sequence of SEQ ID NO:1 in which nucleotide residues 283-285 are changed to TAC, TAT, TTC or TTT;
- (c) the nucleotide sequence of SEQ ID NO:1 in which nucleotide residues 253-255 are changed to AGT, AGC, TCT, TCC, TCA, TCG, ACC, ACA, ACG, ACT, 15 GCC, GCA, GCG or GCT, and nucleotide residues 283-285 are changed to TAC, TAT, TTC or TTT;
- (d) the nucleotide sequence of SEQ ID NO:1 in which nucleotide residues 118-120 are changed to CAG or CAA, and nucleotide residues 283-285 are changed to TAC, TAT, TTC or TTT;
- 20 (e) the nucleotide sequence of SEQ ID NO:1 in which nucleotide residues 118-120 are changed to CAG or CAA, and nucleotide residues 253-255 are changed to AGT, AGC, TCT, TCC, TCA, TCG, ACC, ACA, ACG, ACT, GCC, GCA, GCG or GCT; and
- (f) the nucleotide sequence of SEQ ID NO:1 in which nucleotide residues 118-25 120 are changed to CAG or CAA, nucleotide residues 253-255 are changed to AGT, AGC, TCT, TCC, TCA, TCG, ACC, ACA, ACG, ACT, GCC, GCA, GCG or GCT, and nucleotide residues 283-285 are changed to TAC, TAT, TTC or TTT.

30 21. The nucleic acid according to claim 18, 19 or 20, which is isolated.

22. A recombinant vector comprising the nucleic acid according to claim 18, 19 or 20 operably linked to a promoter.

35 23. A host cell comprising the recombinant vector according to claim 22.

24. A method for producing a modified L49 sFv comprising culturing the host cell of claim 23 such that the nucleic acid is expressed by the cell to produce its encoded modified L49 sFv molecule; and isolating the expressed molecule.

5 25. A pharmaceutical composition comprising (a) a purified nucleic acid according to claim 19; and (b) a pharmaceutically acceptable carrier.

10 26. A method for treating or preventing cancer, wherein the cancer expresses p97 melanotransferrin comprising administering to a subject in need of such treatment or prevention, an effective amount of a pharmaceutical composition according to claim 17 or 25.

15 27. A method for treating or preventing cancer, wherein the cancer expresses p97 melanotransferrin comprising administering to a subject in need of such treatment or prevention, (a) an effective amount of a pharmaceutical composition comprising a purified modified L49-sFv according to any of claims 1-6 fused or conjugated to a pro-drug converting enzyme; and (b) an effective amount of a pro-drug that is converted to its active form by said pro-drug converting enzyme.

20 28. The method according to claim 26 or 27, in which the cancer is a melanoma.

25 29. The method according to claim 26 or 27, in which the cancer is breast cancer, lung cancer, ovarian cancer, renal cancer or colon cancer.

30 30. A method for treating or preventing cancer, wherein the cancer expresses p97 melanotransferrin comprising administering to a subject in need of such treatment or prevention, (a) an effective amount of a pharmaceutical composition comprising a purified modified L49-sFv according to any of claims 1-6 fused to a therapeutic agent, wherein said therapeutic agent is selected from the group consisting of an androgen, anthramycin (AMC), asparaginase, auristatin, 5-azacytidine, azathioprine, bleomycin, busulfan, buthionine sulfoximine, camptothecin, carboplatin, carmustine (BSNU), CC-1065, chlorambucil, cisplatin, colchicine, cyclophosphamide, cytarabine, cytidine arabinoside, cytochalasin B, dacarbazine, dactinomycin, daunorubicin, decarbazine, docetaxel, doxorubicin, an estrogen, 5-fluorodeoxyuridine, 5-fluorouracil, gramicidin D,

hydroxyurea, idarubicin, ifosfamide, irinotecan, lomustine (CCNU), mechlorethamine, melphalan, 6-mercaptopurine, methotrexate, mithramycin, mitomycin C, mitoxantrone, nitroimidazole, paclitaxel, plicamycin, procarbazine, streptozotocin, tenoposide, 6-thioguanine, thioTEPA, topotecan, vinblastine, vincristine, vinorelbine, VP-16 and VM-26.

5

31. A molecule comprising the modified L49-sFv according to any of claims 1-13.

32. A molecule comprising the nucleic acid according to any one of claims 10 18-20.

33. The molecule according to claim 31 or 32 which is purified.

15

20

25

30

35